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# *Gymnosporangium huanglongense* sp. nov. from western China

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Abstract — A novel rust species, *Gymnosporangium huanglongense,* was detected on *Juniperus przewalskii* in China. This species was characterized by the basal cell of its two-celled teliospores possessing two pores near the septum, and the distal cell possessing either two pores near the septum or occasionally an apical pore and one pore near the septum. It was also separated from other *Gymnosporangium* species based on analyses of internal transcribed spacer region and LSU rDNA partial gene sequences.

Key words — *Pucciniales,* Qilian Mountains, taxonomy

## Introduction

The genus *Gymnosporangium* was validated by de Candolle in the Flore Française in 1805, based on a manuscript by R. Hedwig, with *G. fuscum* DC. on *Juniperus sabina* L. as the type species (Kern 1973). *Gymnosporangium* species are mainly distributed in the northern hemisphere. Kern (1973) reported 57 species, and four additional species have subsequently been described (Azbukina 1997, Zhao & Zhuang 2007, Yun et al. 2009). Eighteen species have been recorded from China: 14 with the telial stage on *Juniperus,* two with the telial stage on *Cupressus,* and two with only the aecial stage (Deng 1963, Tai 1979; Wang & Guo 1985; Zhao & Jiang 1986; Cao & Li 1999; Zhuang 2005, 2012; Zhao & Zhuang 2007).

During our investigation of tree diseases in western China, we found a telial stage of *Gymnosporangium* species on *Juniperus przewalskii* Kom., an endemic

TABLE 1. *Gymnosporangium* species and GenBank accession numbers used in this study.

| SPECIES | VOUCHER[*] | GENBANK — ACCESSION NO. | |
|---|---|---|---|
| | | ITS | LSU |
| ***G. huanglongense*** | **BJFC-R01984** | **KT719167** | **KT719161** |
| | **BJFC-R01985** | **KT719168** | **KT719162** |
| *G. amelanchieris* E. Fisch. ex F. Kern | 20140331-1 M1-M3[a] | KM48$^{6}$547b | — |
| *G. asiaticum* Miyabe ex G. Yamada | BJFC-R01834 | KR814568 | KT719165 |
| | BJFC-R01835 | KR814569 | KT719166 |
| | HKFRI 1974 | — | FJ848741[b] |
| | HKFRI 1976 | — | FJ848743[b] |
| *G. clavariiforme* (Wulfen) DC. | ML 841[a] | — | AF426$^{2}$11b |
| | BPI 880006 | — | HM114220[b] |
| *G. clavipes* Cooke & Peck | DAOM 234635 | HQ317507[b] | — |
| *G. confusum* Plowr. | BPI 879272 | — | GU058011[b] |
| | BPI 880005 | — | HM114219[b] |
| *G. cornutum* Arthur ex F. Kern | HKFRI 2105 | — | FJ848766[b] |
| | WM 1093 | — | AF426210[b] |
| *G. fusisporum* E. Fisch. | HMAS 52880 | — | KJ720172[b] |
| *G. globosum* (Farl.) Farl. | DAOM 234634 | HQ3175$^{0}$6b | — |
| | DAOM 234636 | HQ317511[b] | — |
| *G. gracile* Pat. | 20140326-1-GR-P25[a] | KM4865$^{4}$2b | — |
| | 20140529-1a[a] | KM4865$^{4}$3b | — |
| *G. japonicum* Dietel & P. Syd. | HKFRI 1992 | — | FJ848755[b] |
| | HKFRI 1993 | — | FJ848756[b] |
| *G. juniperi-virginianae* Schwein. | DAOM 234434 | HQ317510[b] | — |
| | PBM 2530 | DQ2671$^{2}$7b | — |
| | TDB1345[a] | — | AF5$^{2}$2167b |
| | BPI 871103 | — | DQ354547[b] |
| *G. monticola* H.Y. Yun | HKFRI 1984 | — | FJ848770[b] |
| | HKFRI 1985 | — | FJ848771[b] |
| *G. nidus-avis* Thaxt. | RSP 05-29[a] | — | KJ720$^{1}$81b |
| *G. sabinae* (Dicks.) G. Winter | BG5[a] | KF925$^{3}$20b | — |
| | BG6[a] | KF9253$^{2}$1b | — |
| | WM 1347 | — | AF4262$^{0}$9b |
| | BPI 880007 | — | HM114221[b] |
| *G. unicorne* H.Y. Yun | HKFRI 1971 | — | FJ848767[b] |
| | HKFRI 1972 | — | FJ848768[b] |
| *G. yamadae* Miyabe ex G. Yamada | BJFC-R01822 | KR814566 | KT719163 |
| | BJFC-R01823 | KR814567 | KT719164 |
| | HKFRI 1998 | — | FJ848761[b] |
| | HKFRI 2007 | — | FJ848765[b] |
| *Puccinia graminis* Pers. | K(M) 92038 | AF4680$^{4}$4b | — |
| | ECS[a] | — | AF5221$^{7}$7b |

[*] BJFC: Museum of Beijing Forestry University, Beijing, China; HKFRI: Herbarium of Korea Forest Research Institute, Seoul, Korea; DAOM: Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada; PBM: Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Thailand; BPI: US National Fungus Collections, Maryland, USA; WM: Agricultural Research Division, Gezira Research Station, Wad Medani, Sudan; HMAS: Herbarium Mycologicum Academiae Sinicae, Beijing, China; K: Royal Botanic Gardens, Kew, England.

[a] private collection no.

[b] Sequence downloaded from GenBank.

evergreen conifer, mainly distributed in the forest regions of the Qilian Mountains (Liu et al. 2006). No *Gymnosporangium* species has previously been reported on *J. przewalskii*. Because morphological observations and phylogenetic analyses confirmed that the fungus is distinct from other *Gymnosporangium* species, we describe it here as new.

## Materials & methods

### Specimens

The fungus-infected branches were collected in Qinghai and Sichuan provinces in China, and their herbarium specimens were deposited in the Mycological Herbarium, Museum of Beijing Forestry University, Beijing, China (BJFC).

### Morphological observation

Teliospores were scraped from the herbarium specimens and mounted in a drop of lactophenol-cotton blue solution. The slide preparations were observed under a Leica DM2500 microscope. The length, width, and wall thickness of 30–50 teliospores from each specimen were measured using a MicroView MVC TWAIN Image Analyzer.

### Phylogenetic analysis

Whole genomic DNA from the teliospores was extracted following the methods described by Tian et al. (2004). DNA concentrations were estimated by electrophoresis in 1.2% agarose gels. The D1/D2 region of nuclear LSU rDNA was amplified with the primer pair No. 4 (5′-ACCCGCTGAAYTTAAGCATAT-3′) and No. 11 (5′-CTCCTTGGTCCGTGTTTCAAGACGC-3′) (Van der Auwera et al. 1994). The ITS and 5.8S region of rDNA were amplified with the primer pair ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) (White et al. 1990) and ITS4B (5′-CAGGAGACTTGTACACGGTCCAG-3′) (Gardes & Bruns 1993). Amplifications were performed in 25 µl of PCR solution containing 1 µl of DNA template, 1 µl of sense primer (10 µM), 1 µl of antisense primer (10 µM), 12.5 µl of 2×Es Taq MasterMix (Cwbio, Beijing, China), and 9.5 µl of ddH$_2$O. The PCR conditions were as follows: 95°C for 3 min, 35 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min, and a final step of 72°C for 10 min. PCR products were purified and cloned for sequencing (Invitrogen, Beijing, China). A sequence of *Puccinia graminis* obtained from GenBank was used as outgroup (Yun et al. 2009).

The sequences newly obtained in this study and additional sequences obtained from GenBank are listed in Table 1. Sequences were aligned using ClustalX (Thompson et al. 1997). The sequence alignment has been deposited at TreeBase (http://www.treebase.org/) under the accession number 17721. Maximum parsimony (MP) analysis was carried out using the heuristic search option with 1,000 random-addition sequences and tree bisection and reconnection as the branch-swapping algorithm implemented in PAUP v.4.0b10 (Swofford 2002). In the MP analyses, gaps were treated as missing data, and all characters were equally weighted. Clade stability was assessed using a bootstrap analysis with 1,000 replicates (Felsenstein 1985). Other measures calculated were tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency (RC). Bayesian analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck

2003) using the Markov Chain Monte Carlo (MCMC) method; the GTR model was used with gamma-distributed rate variation, and the Markov chains were run for 1,000,000 generations. The trees were sampled every 100 generations, producing 10,000 total trees.

TABLE 2. Comparison of telium habit and teliospore morphology between *Gymnosporangium huanglongense* and its morphological allies.

| SPECIES | TELIA | | TELIOSPORE | |
|---|---|---|---|---|
| | POSITION | SHAPE | SIZE (μm) | GERM PORES |
| *G. huanglongense* | caulicolous | conic or irregular | 53–100 × 14–25 | 2/cell, near septum (or 1 apical + 1 near septum in distal cell) |
| *G. clavariiforme*[a,b,c] | caulicolous | terete or slightly compressed | 38–105 × 12–23 | 2/cell, near septum |
| *G. fusisporum*[b,d] | caulicolous | conic or laterally compressed | 46–100 × 16–27 | 1(–2)/cell, near septum |
| *G. gracile*[b,e] | caulicolous | terete | 45–90 × 14–20 | 2/cell, near septum |

References: [a] Parmelee (1965); [b] Kern (1973); [c] Yun et al. (2009); [d] Zhuang (2012); [e] Fischer (1930).

## Taxonomy

### ***Gymnosporangium huanglongense*** Y.M. Liang & B. Cao, **sp. nov.** PLATE 1

MYCOBANK MB 814684

Differs from *Gymnosporangium fusisporum* by the basal cell of its two-celled teliospores possessing two pores near the septum, and the distal cell possessing either two pores near the septum or occasionally an apical pore and one pore near the septum.

TYPE: China, Sichuan Prov., 32°45′06″N 103°48′12″E, alt. 3316 m, on *Juniperus przewalskii*, 24 Apr 2015, B. Cao & X.L. Fan (**Holotype**, BJFC-R01984; GenBank KT719167, KT719161).

ETYMOLOGY: The specific epithet *huanglongense* refers to type locality of this species.

Spermogonia, aecia, and uredinia not found. Telia caulicolous, with little or no hypertrophy on the smaller branches, often conic or irregular, 1–3 mm high, brown to chestnut-brown; teliospores two-celled, rarely one-celled, fusiform, usually narrowed above and below, more or less constricted at the septum, yellowish brown, 53–100 × 14–25 μm; walls 0.7–1.7 μm thick; the basal cell with two pores near the septum, and the distal cell with two pores either both near the septum or occasionally (c. 3%) one apical and one near the septum; pedicels ≤200 μm long.

OTHER SPECIMENS EXAMINED: On *Juniperus przewalskii*: CHINA, SICHUAN PROV., 32°45′06″N 103°48′12″E, alt. 3307 m, 24 Apr 2015, B. Cao & X.L. Fan (BJFC-R01982; BJFC-R01985, GenBank KT719168, KT719162); QINGHAI PROV., 35°18′33″N 101°56′30″E, alt. 2934 m, 7 Apr 2015, F. Z. Han (BJFC-R01965; BJFC-R01966).

COMMENTS: The telia of the new species are caulicolous and scattered, often conic or irregular, and *J. przewalskii* was not previously known as a

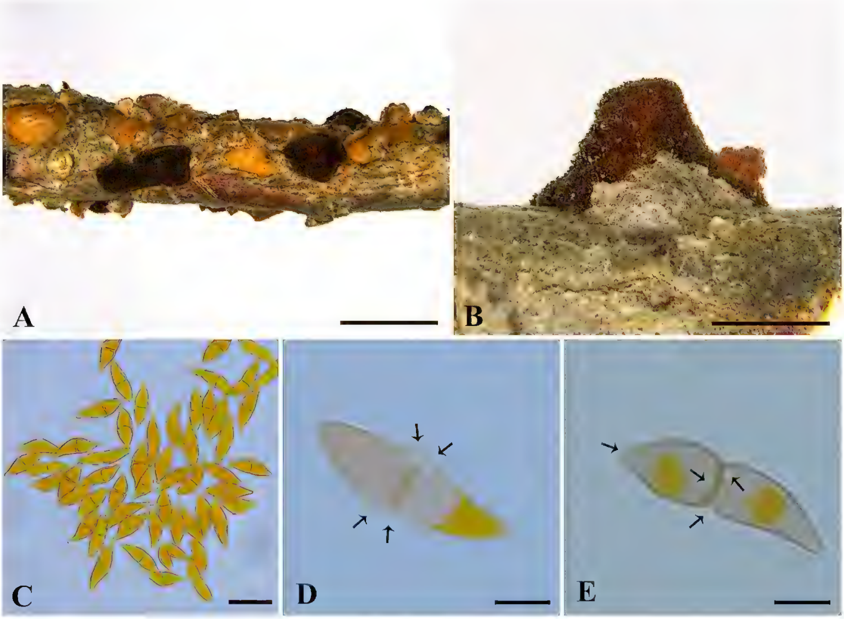

PLATE 1. *Gymnosporangium huanglongense* (holotype, BJFC-R01984). A. telia formed on a branchlet; B. an enlarged view of a telium; C. teliospores. D. teliospore with two septal germ pores in each cell (arrows); E. teliospore with septal and apical germ pores (arrows). Scale bars: A, B = 3 mm; C = 70 µm; D, E = 20 µm.

*Gymnosporangium* host. The new species is morphologically similar to three *Gymnosporangium* species, *G. clavariiforme*, *G. fusisporum*, and *G. gracile*, but *G. huanglongense* can be distinguished from them by morphological characteristics (TABLE 2). *Gymnosporangium clavariiforme* and *G. gracile* differ by their terete telia (Fischer 1930, Parmelee 1965, Kern 1973, Yun et al. 2009), and *G. fusisporum* differs by its teliospore cells that have only one (rarely 2) pores near the septum (Kern 1973, Zhuang 2012). Additionally, the phylogenetic analyses supports *G. huanglongense* as distinct from these three species (PLATES 2, 3).

The aligned ITS dataset from 16 ingroup taxa including nine terminal clades consisted of 713 characters, of which 311 were parsimony-informative, 322 were invariant, and 80 variable characters were parsimony-uninformative. The MP analysis of sequence data yielded a single parsimonious tree (TL = 757, CI = 0.745, RI = 0.800, and RC = 0.596). Bayesian analysis resulted in the same topology with an average standard deviation of split frequencies of 0.006386. In the LSU rDNA phylogenetic analyses, 28 ingroup taxa clustered into thirteen

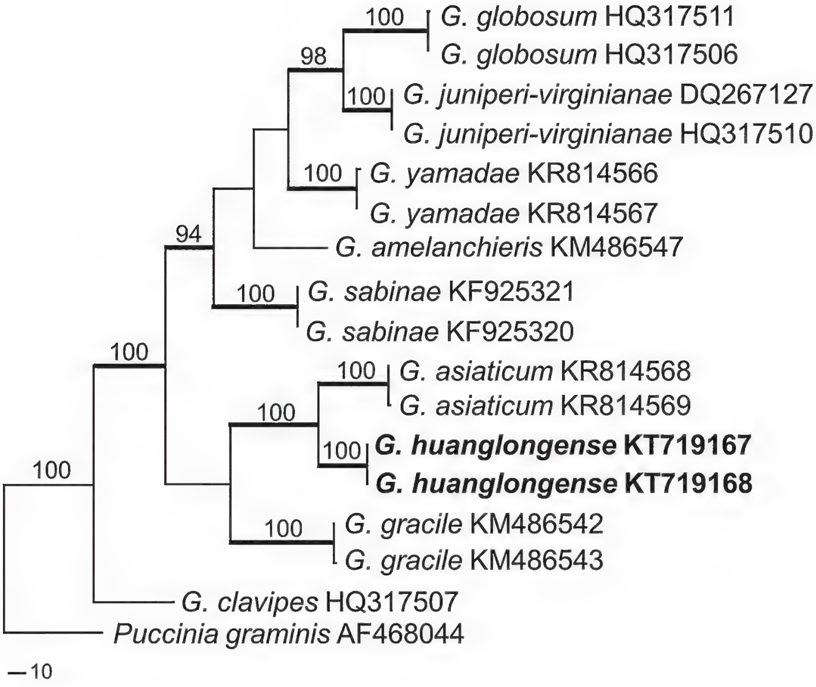

Plate 2. ITS phylogenetic tree of *Gymnosporangium* spp., constructed by maximum parsimony and Bayesian methods. Node support is presented as: parsimony bootstrap values >90%. Thickened branches indicate PP > 0.90 from the Bayesian inferences. The new species is shown in bold.

terminal clades. The final aligned dataset consisted of 593 characters of which 501 were invariant. MP analyses of the remaining 54 parsimony-informative characters resulted in 20 trees. We selected the tree with the shortest length (TL = 140, CI = 0.743, RI = 0.865, and RC = 0.642) as the current tree. Bayesian analysis resulted in an average standard deviation of split frequencies of 0.010532. The topology of the ITS phylogram was consistent with the tree estimated using the LSU rDNA region (Plates 2, 3), indicating that the *G. huanglongense* sequences were distinct from those of other species which are available in GenBank database.

In the LSU rDNA phylogenetic analyses, specimens of *G. huanglongense* formed a distinct lineage with high support values (97 MPBS; 0.99 BPP). The other four species, *G. asiaticum*, *G. unicorne*, *G. confusum*, and *G. fusisporum*

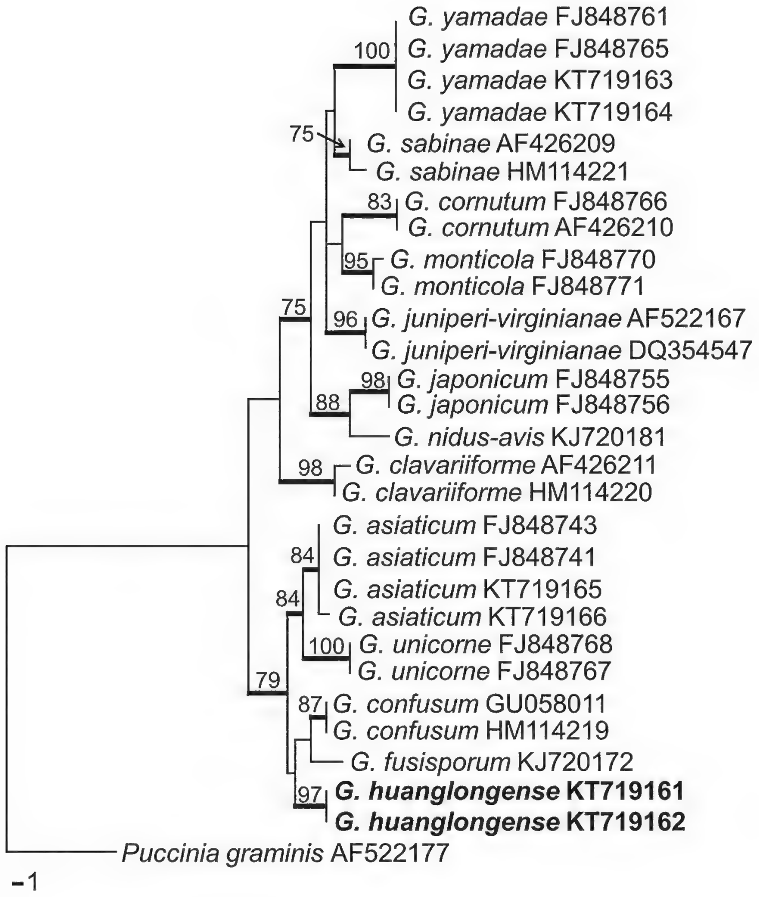

PLATE 3. LSU phylogenetic tree of *Gymnosporangium* spp., constructed by maximum parsimony and Bayesian methods. Node support is presented as: parsimony bootstrap values >75%. Thickened branches indicate PP > 0.75 from the Bayesian inferences. The new species is shown in bold.

were closely related to *G. huanglongense* based on LSU rDNA analyses (79 MPBS; 0.87 BPP). However, these four species differ morphologically from *G. huanglongense*. As previously discussed, *G. fusisporum* differs by its smaller number of teliospore germ pores; and the other three species differ by their shorter teliospores: *G. asiaticum* 35–45 µm; *G. confusum* 35–48 µm; and *G. unicorne* 31–47 µm (Kern 1973, Yun et al 2009).

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